

REMARKS

Claims 1, 4, 5, 12-19, 21, 24, 25, 32-39, 41, 42, 56, 57, 59 and 89-91 stand rejected in the present application. Applicants reserve the right to prosecute any canceled subject matter at a later date or in a timely filed divisional application.

Claims 1, 4, 21, 24, 32, 39, 56, and 57 have been amended. New claims 63 and 64 have been added. Support for the amendments and new claims can be found throughout the specification as filed originally, for example at page 20, line 30 et seq.; page 4, line 34 et seq.; Figure 4; Figure 5; Example 7, Example 8. Support for the amended claims can be found throughout the application as filed originally, for example at page 5, lines 11-27, page 6, lines 26-29, page 11, lines 6-11, Examples 1, 8, 10, 11, and 13. No new matter is introduced by these amendments.

Rejections under 35 U.S.C. § 112

The Examiner, on pages 3-4 of the Office Action mailed April 1, 2009, rejects claims 1, 4-5, 12-19, 21, 24-25, 32-39, 41-42, 56-57, 59 and 89-91 under 35 U.S.C. § 112, first paragraph “as failing to comply with the written description requirement.” Applicants traverse the rejection.

The amended claims provide for a narrow genus of chemical compounds defined both by structure and in function as binding to F36V. Moreover, on page 55, lines 31-32, of the instant specification, the Applicants incorporate by reference “each of the patent documents and scientific papers identified herein is hereby incorporated by reference.” Then, on page 37, lines 5-7, of the present specification, Applicants incorporate the patent documents WO 96/06097 and WO 97/31898, noting that a “variety of other synthetic dimerizing agents are disclosed in WO 96/06097 and WO 97/31898 for binding to FKBP-related domains.” These publications, which are reflected in the Holt patent cited by the Examiner with respect to § 103, discussed below, provide support that the Applicants were in possession of the claimed invention at the time of filing.

The court has instructed that when structure is readily accessible on the internet to those of skill in the art, as provided by Applicants’ reference to the incorporated and publicly available WO applications, structure need not be reiterated in the specification. Literature sources may be relied on to provide structure. *Capon v. Eshhar*, 418 F.3d 1349 (2005). In *Falkner v. Inglis*, (Fed.

Cir. 2006), the court held:

Specifically, we hold, in accordance with our prior case law, that (1) examples are not necessary to support the adequacy of a written description (2) the written description standard may be met (as it is here) even where actual reduction to practice of an invention is absent; and (3) there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure.

Moreover, the court's precedent does not require a re-description of what was already known. *Id.* The forced recitation of the structures already published in the incorporated WO applications would only add unnecessary bulk to the specification. As in the *Falkner* case, the instant specification and accessible literature sources provided, as of the filing date, chemical structure of the claimed divalent ligand in satisfaction of the written description requirement. Indeed, given the ready accessibility of the chemical structure of the claimed compound, the absence of incorporation by reference would not be problematic. See *Faulkner*, at slip. op. 12. Applicants respectfully request that this § 112 rejection be withdrawn.

On page 6 of the Action, the rejects claims 1, 4-5, 12-19, 21, 24-25, 32-39, 41-42, 56-57, 59 and 89-91 under 35 U.S.C. § 112, second paragraph "as being indefinite." Applicants traverse the rejection. Regarding the structures of the characters represented by X, G, etc., the claims have been amended to clarify these moieties.

The Examiner further found that claim 21 is indefinite in lacking antecedent basis for "said transduced cells." This claim has been amended to recite "a subpopulation of mammalian primary hematopoietic stem cells which has been transduced with at least one retroviral vector."

The Examiner found claim 24 indefinite in lacking antecedent basis for "the population" as opposed to "sub-population;" this has been amended accordingly.

The Examiner held claim 32 is indefinite in lacking antecedent basis because it depends from 21, but refers to "the transduction thereof with the retroviral vector," but claim 21 recites "providing a subpopulation of mammalian primary hematopoietic stem cells which contain a retroviral vector ..." but does not recite the step of transducing the cell, thus claim 32 has no antecedent basis for transducing cells in claim 32. Examiner is unclear whether applicants intend to add the step of transducing cells to the method of claim 21. Claim 32 has been amended to clarify that "the subpopulation of mammalian primary hematopoietic stem cells which has been transduced with the at least one retroviral vector was transduced *ex vivo*."

Similarly, the Examiner found that claim 39 is indefinite in lacking antecedent basis because it recites “wherein the cells are transduced within the mammal,” but depends on claim 21 which does not contain this step. Claim 39 has been amended to clarify that the subpopulation of mammalian primary hematopoietic stem cells which has been transduced with the at least one retroviral vector was transduced within the mammal.

The Examiner also found that that claim 56 is indefinite because it apparently recites a method of using the product of either claim 4 or 24 which refer to “populations primary hematopoietic cells comprising...” but not to bone marrow cells, cord blood cells or peripheral blood cells themselves. Claim 56 has been amended to clarify that the method uses the subpopulation of cells of claim 4 or 24. To further prosecution of the instant claims, Applicants have addressed the indefiniteness issues by amendment. Therefore, these § 112 rejections may be withdrawn.

Rejections under 35 U.S.C. § 103(a)

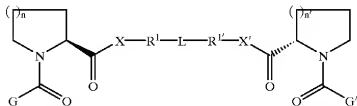
The Examiner, on page 8 of the Office Action, the Examiner rejects claims 1, 4-5, 12-19, 21, 24-25, 32-30, 41-42, 56-57, 59, and 89-91 “as being unpatentable over U.S. Patent No. 5,741,899 ... Capon et al., in view of Blau et al. (1996) Blood, ... meeting abstract ..., Ramsfjell et al. (1996) Blood, Vol. 88(12), 4481-4492, and U.S. Patent No. 6,150,527 (2000), hereinafter referred to as Holt et al.” Applicants traverse the rejection.

At the outset, the case *In re O'Farrell* is instructive on the present rejections:

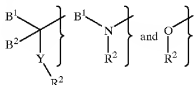
The admonition that “obvious to try” is not the standard has been directed mainly at two kinds of error. In some cases, *what would have been “obvious to try” would have been to vary all parameters or to try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many choices were likely to be successful.* ... In others what was “obvious to try” was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave *only general guidance* as to the *particular form* of the claimed invention or how to achieve it. *In re O'Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988) (emphasis added).

The claimed invention relates to methods for rendering a subpopulation of mammalian primary hematopoietic stem cells susceptible to divalent ligand-induced growth, proliferation or differentiation. More specifically, the claimed invention provides for methods for rendering a subpopulation of mammalian hematopoietic stem cells susceptible to divalent ligand-induced

growth, proliferation or differentiation, by transducing mammalian primary hematopoietic stem cells with at least one retroviral vector that carries a recombinant DNA construct encoding a fusion protein with at least one signaling domain derived from an intracellular portion of a thrombopoietin receptor and at least one ligand-binding domain derived from F36V that binds to a divalent ligand capable of inducing association of two or more molecules of F36V (e.g., AP1903, AP1510 or AP20187), such that upon exposure of the transduced cells to a concentration of the divalent ligand having the formula:

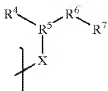


effective to induce association of two or more of the encoded fusion proteins, growth, proliferation or differentiation of said cells is induced; wherein X and X' can be O, NH, or CH₂; L is a covalently linker moiety; G and G' are independently selected from the group comprised of



in which B¹ and B² are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, aryl, or heteroaryl moieties; R¹, R^{1'}, and R² are the same or different and are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkyl, aryl, or heteroaryl moieties; and n and n' are each independently 1 or 2;

wherein at least one of X-R¹ and X'-R^{1'} is independently a moiety:



wherein R⁴ is hydrogen; branched, unbranched, cyclic, saturated or unsaturated, substituted or unsubstituted aliphatic; branched, unbranched or cyclic heteroaliphatic; aryl or heteroaryl; R⁵ is

a branched, unbranched or cyclic, aliphatic moiety of 1 to 8 carbon atoms; R⁶ is a substituted or unsubstituted aliphatic, heteroaliphatic, heterocyclic, aryl or heteroaryl moiety; and R⁷ is hydrogen or a reactive functional group permitting covalent attachment to a linker moiety; and wherein the transduction is carried either out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal.

The claimed invention thus provides for pharmacologically-responsive fusion proteins that are useful for specifically and reversibly expanding genetically modified primary hematopoietic stem cell populations, which populations may be differentiated. This allows, for the first time, application of a neutral, targeted approach that does not rely on either negative selective pressure (e.g., antibiotic resistance) or addition of a growth factor that might have systemic implications, to provide selective positive inducement for cell growth, proliferation and/or differentiation.

Returning to the cited combination of references, on pages 8-9 of the Office Action, the Examiner asserts that:

Capon et al. teaches that the extracellular or intracellular inducer-responsive clustering domain (ICD) of the chimeric protein is derived from immunophilin, e.g., FKBP, and that the cytoplasmic signal transduction domain is derived from homodimerizing receptors such as TPOR (thrombopoietin receptor or mpl) (Capon et al., columns 7, 9, 13, 15, 34-35, and 42-43).

In doing so, the Examiner has focused on only 1 of numerous such domains identified in Capon. For example, regarding the inducer-responsive clustering domain, Capon recites, *inter alia*:

The intracellular clustering domain (ICD) can be obtained from the inducer binding domains of a variety of intracellular proteins. For example, eukaryotic steroid receptor molecules can be used as ICDs (e.g. the receptors for estrogen, progesterone, androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid and ecdysone). In addition, variants of steroid and other receptors which fail to bind their native inducer, but still bind to an antagonist, can be prepared by one skilled in the art and used to make the CPRs of this invention. For example, a C-terminal deletion mutant of the human progesterone receptor, which fails to bind progesterone, can be clustered by the addition of progesterone antagonists, including RU 486. Binding domains from the eukaryotic immunophilin family of molecules may also be used as ICDs. Examples include but are not limited to members of the cyclophilin family: mammalian cyclophilin A, B and C, yeast cyclophilins 1 and 2, *Drosophila* cyclophilin analogs such as ninaA; and members of the FKBP family: the various mammalian isoforms of FKBP and the FKBP analog from *Neurospora*. For example, the inducer binding portion of the immunophilin, FKBP12, which can be clustered in the cytoplasm by the addition of FK1012, a synthetic dimeric form of the immunosuppressant FK506 can be used as an ICD. Col. 14, line 55-col. 14, line 16 (internal citations omitted).

In this paragraph alone, Capon refers to **at least 20** ICD domains, including receptors for estrogen, progesterone, androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid and ecdysone, a C-terminal deletion mutant of the human progesterone receptor, mammalian cyclophilin A, cyclophilin B and cyclophilin C, yeast cyclophilins 1 and 2, *Drosophila* cyclophilin analogs such as ninaA, various mammalian isoforms of FKBP and the FKBP analog from *Neurospora*, and FKBP12.

Regarding the cytoplasmic signal transduction domain, Capon recites, *inter alia*:

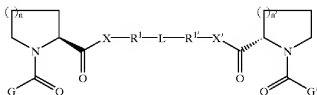
The proliferation signaling domains (PSDs) that comprise the chimeric proliferation receptors (CPRs) of the present invention (both CIPRs and CEPRs) may be obtained from the cytoplasmic signal-transducing domains of the cytokine/hematopoietin receptor superfamily. The members of this mammalian receptor superfamily can transduce proliferative signals in a wide variety of cell types. These receptors are structurally related to each other. The cytoplasmic domains of the signal-transducing subunits may contain conserved motifs that are critical for transduction of proliferative signals. In contrast to the growth factor receptors previously described in chimeric receptors, the cytoplasmic portions of the cytokine receptor superfamily proteins that comprise the PSDs employed in the present invention do not contain any kinase domains or other sequences with recognizable catalytic function. Further, although the growth factor receptors described by Ullrich and the cytokine receptors employed in the present invention both dimerize upon binding of inducer, the dimerized growth factor receptors activate their intrinsic receptor kinase activity, while the dimerized cytokine receptors employed in the present invention stimulate the activity of associated tyrosine kinases. The signal-transducing components of the cytokine receptors to be used in the PSDs of the present invention include, but are not limited to, Interleukin-2 receptor β (IL-2R β), IL-2R γ , IL-3R β , IL-4R, IL-5R α , IL-5R β , IL-6R, IL-6R, gp130, IL-7R, IL-9R, IL-12R, IL-13R, IL-15R, EPO-R (erythropoietin receptor), G-CSFR (granulocyte colony stimulating factor receptor), GM-CSFR α (granulocyte macrophage colony stimulating factor receptor α), GM-CSFR β , LIFR α (leukemia inhibitory factor receptor α), GHR (growth hormone receptor), PRLR (prolactin receptor), CNTFR (ciliary neurotrophic factor receptor), OSMR (oncostatin M receptor) IFNR α/β (interferon α/β receptor), IFNR γ , TFR (tissue factor receptor), and TPOR (thrombopoietin or mpl-ligand receptor). Col. 8, line 40-col. 9, line 20 (internal citations omitted.)

Col. 9, to which the Examiner refers, recites at least IL-2R β , IL-2R γ , IL-3R β , IL-4R, IL-5R α , IL-5R β , IL-6R, gp130, IL-7R, IL-9R, IL-12R, IL-13R, IL-15R, EPO-R, G-CSFR, GM-CSFR α , GM-CSFR β , LIFR α , GHR, PRLR, CNTFR, OSMR, IFNR α/β , IFNR γ , TFR, and TPOR, providing **at least 26** proliferation domains to the list of possible domains. Cols. 34-35 and 42-43, to which the Examiner refers, add numerous JAK receptors.

In just these two presented paragraphs, Capon presents *over 500 possible chimeric fusion proteins*, providing no expectation of which combination of these parameters would render a subpopulation of mammalian hematopoietic stem cells susceptible to drug-induced growth,

proliferation or differentiation upon exposure to the claimed divalent ligand. The Capon Examples refer to FKBP in the context of JAK, IL-2, or glucocorticoid receptor, and the Examples refer to **neither** the F36V, **nor** the divalent ligand of the claimed invention. In reference to the “saturating concentrations” of FK1012 cited by the Examiner in cols. 42-43, this has been the subject of previous discussion and Declaration regarding the toxicity of such treatment, and need not be repeated here although it is incorporated herein by this reference. The Examiner’s selection of 2 of the literally hundreds of Capon parameters and possible choices, in total disregard of these alternatives suggested equally by Capon, is a simple exercise of hindsight. Capon simply does not teach or suggest the particular claimed chimeric protein derived from thrombopoietin receptor and FKBP_{F36V}.

Moreover, the transduction of the primary hematopoietic stem cells with the chimeric receptor requires, according to the claimed methods, exposure to a concentration of a specific divalent ligand having the structure



effective to induce association of two or more fusion proteins, thereby inducing growth, proliferation or differentiation of said cells. In that regard, Capon presents a myriad of inducer drugs in addition for FK1012, for example, *inter alia*:

Examples of inducers include, but are not limited to synthetic dimeric molecules such as FK1012 or dimeric derivatives of the binding domains of other immunophilin binding molecules such as cyclosporin, rapamycin and 506BD. Steroids, such as estrogen, progesterone, the androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid or ecdysone, or antagonists or derivatives of these molecules may also be used as intracellular inducer molecules. In particular the steroid antagonist RU 486 may be used. Col. 15, lines 24-35 (internal citations omitted).

Extracellular inducers of the present invention can be antigens which bind the ECDs, described above. These may include viral proteins, (e.g. gp120 and gp41 envelope proteins of HIV, envelope proteins from the Hepatitis B and C viruses, the gB and other envelope glycoproteins of human cytomegalovirus, the envelope proteins from the Kaposi's sarcoma-associated herpesvirus), and surface proteins found on cancer cells in a specific or amplified fashion, (eg the IL-14 receptor, CD19 and CD20 for B cell lymphoma, the Lewis Y and CEA antigens for a variety of carcinomas, the Tag72 antigen for breast and colorectal cancer, EGF-R for lung cancer, and the HER-2 protein which is often amplified in human breast and ovarian carcinomas). For other receptors, the receptors and ligands of particular interest are CD4, where the ligand is the HIV gp120 envelope

glycoprotein, and other viral receptors, for example ICAM, which is the receptor for the human rhinovirus, and the related receptor molecule for poliovirus. Col. 14, lines 37-54 (internal citations omitted).

The Examiner states that “In regards to cells transduced *ex vivo* and introduced into a host mammal, Capon teaches that the cells can be allogenic or autologous cells, including hematopoietic stem cells capable of developing into cells of the myeloid and lymphoid lineages (...columns 26, and 21-22).” Once again, the Examiner has employed hindsight to pick one term out of numerous alternatives suggested by Capon:

The CPRs of the present invention are employed in a *wide variety of target host cells*, normally cells from vertebrates, more particularly, mammals, desirably domestic animals or primates, particularly humans. In particular, the subject invention may also find application in the *expansion of lymphoid cells, e.g., T lymphocytes, B lymphocytes, cytotoxic lymphocytes (CTL), natural killer cells (NK), tumor-infiltrating-lymphocytes (TIL) or other cells which are capable of killing target cells when activated*. In addition, suitable host cells to introduce CPRs of the present invention include hematopoietic stem cells, which develop into cytotoxic effector cells with both myeloid and lymphoid phenotype including granulocytes, mast cells, basophils, macrophages, natural killer (NK) cells and T and B lymphocytes. In particular, *diseased cells*, such as cells infected with HIV, HTLV-I or II, cytomegalovirus, hepatitis B or C virus, *Mycobacterium avium*, etc., neoplastic cells, or autoimmune disease-causing cells where the diseased cells have a surface marker associated with the diseased state may be made specific targets of the cells expressing the CPRs of the present invention. In the present invention, a cell may express dual CEFR and CPR receptors, which contain the same extracellular binding domain (eg. CD4), or a cell may express a hybrid chimeric receptor combining both signaling domains (EFSD and PSD). In each case, the binding of one inducer to the extracellular binding domain will stimulate cells to act as therapeutic agents at the same time they are expanding in response to binding to inducer, e.g., gp120 for HIV or cancer-specific antigens. Col. 16, lines 7-35 (emphasis added)

In a preferred embodiment, the present invention relates to the design of chimeric proliferation receptor (CPR) molecules which can endow *T cells* with the ability to proliferate in an antigen-specific and IL-2 independent manner....

In another preferred embodiment, the present invention relates to the use of chimeric proliferation receptors to induce the proliferation of *T cells*, where the proliferation signaling domains are comprised of one or more of the family of Janus kinases, i.e., JAK1, JAK2, JAK3, Tyk2 and Ptk-2....

In yet another preferred embodiment, the present invention relates to T cells containing single chimeric polypeptide receptors that drive both proliferation and effector function through the same inducer molecule....

In yet another preferred embodiment, the present invention relates to engineered T cells expressing CPRs which already contain a chimeric effector function receptors....

Other cell types that would be of particular interest for expansion after delivery of the CPRs of the subject invention are *islets of Langerhans* which may be grown and introduced into a host by capsules or other means, for the production of insulin. *Retinal epithelial cells* may also be expanded and injected or implanted into the subretinal space of the eye to treat visual disorders, such as macular degeneration. Immune cells, described in detail above, may be expanded *ex vivo* and injected into the bloodstream or elsewhere to treat immune deficiency. *Myoblasts* may be

expanded with the present invention and injected at various sites to treat muscle wasting diseases such as Duchenne muscular dystrophy. **Hepatocytes** may be expanded for use in liver regeneration. **Endothelial cells** may also be expanded to repair blood vessels or to deliver proteins to the circulation. **Nerve cells** which ordinarily do not proliferate may be targets for expression by using the CPRs of present invention. In addition cells which will not proliferate *in vitro*, and therefore cannot be manipulated or genetically engineered may be ideal recipients of the CPRs of the present invention. Col. 21, lines 16-35 (emphasis added).

A thorough reading of Capon shows that the primary target of Capon is T-cells, including engineered T-cells expressing dual chimera receptors to be activated at disease cite (col. 18, lines 9-12); cytotoxic T-cells that recognize HIV antigens (col. 18, lines 15-19). Additional cell types include lymphocytes targeting neoplastic cells, virus-infected cells, parasite-infected cells, or any other diseased cells (col. 20, lines 16-21); cytotoxic CD8⁺ T-cells against CMV, HIV, Hepatitis B virus, Hepatitis C virus, Kaposi's sarcoma-associated Herpes virus, Herpes Simplex virus, Herpes Zoster virus, and papilloma viruses, neoplastic cells, IL-14 receptor, CD19 and CD20 for B cell lymphoma, the Lewis Y and CEA antigens for a variety of carcinomas, Tag72 antigen for breast and colorectal cancer, EGF-R for lung cancer, human Heregulin (Hrg), or against autoimmune cells in the treatment of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), myasthenia gravis, diabetes, rheumatoid arthritis, and Grave's disease (col. 20, lines 22-60); CD4⁺ helper T-cells against cancer cells and mycobacterial infections, including *Mycobacterium avium*, *Mycobacterium tuberculosis* and *Mycobactium leprae* (col. 20, lines 61-67); "various cell types" to reconstruct existing tissue or provide new tissue in transplantation therapy, for example, keratinocytes for skin (col. 21, lines 1-15); islets of Langerhans, "immune cells," myoblasts, hepatocytes, endothelial cells, nerve cells (col. 21, lines 16-35); and "additional cell types" to correct gene defects or produce proteins (col. 21, lines 36-54). Capon thus refers to numerous cell targets, with no particular teaching why hematopoietic stem cells should be transduced with the chimeric construct of the present claims. Interestingly, and perhaps tellingly, Capon is silent regarding the generation of erythrocytes.

The Examiner states, on page 9 of the Action, that Capon "does not specifically exemplify ligand induced proliferation of hematopoietic stem cells expressing a chimeric protein comprising FKBP and TPOR," and asserts that the Blau abstract supplements Capon "by teaching that cell proliferation of hematopoietic stem cells can be effectively induced through dimerization of chimeric receptors comprising FKBP and EpoR using FK1012." This is simply incorrect.

The Blau abstract states:

A DRUG DEPENDENT PROLIFERATIVE SWITCH FOR GENETICALLY MODIFIED CELLS
C.A. Blau,* KIL Peterson,* J.G. Drachnean, and D. Spencer* University of Washington, Seattle, WA, and Baylor College of Medicine, Houston, TX

Receptor dimerization is the key signaling event for many cytokines, including erythropoietin. A system has been recently developed which permits intracellular protein dimerization to be reversibly activated in response to a lipid soluble dimeric form of the drug FK506, called FK1012. FK1012 is used as a pharmacological mediator of dimerization to bring together two FK506 binding domains, taken from the endogenous protein FKBP12. Experiments were performed to determine whether FK1012 could be used to reversibly activate cell proliferation. The murine IL3 dependent cell line Ba/F3 was used as a model system. Previous studies have shown that forced expression of the erythropoietin receptor in Ba/F3 cells allows for erythropoietin-driven proliferation. Three Ba/F3 clones expressing high levels of a membrane targeted fusion protein were obtained. The fusion protein consisted of a myristylation targeting domain, three FKBP12 repeats, the 236 amino acid cytoplasmic domain of the erythropoietin receptor, and an epitope tag. After IL3 was withdrawn, each clone exhibited a dose-dependent proliferative response to FK1012. Each clone remained dependent on either IL3 or FK1012 for survival. FK506 competitively reversed the proliferative effect of FK1012, but had no influence on the proliferative effect of IL3. Signaling pathways appeared to mimic those activated by erythropoietin, as FK1012 induced β -globin mRNA expression. Five control clones which expressed a fusion protein lacking the erythropoietin receptor component failed to proliferate in response to FK1012. FK1012-dependent clones were also generated using a fusion protein containing only the membrane proximal portion of the erythropoietin receptor, in which 133 amino acids of the carboxy-terminus had been deleted. A fusion protein containing only a single FKBP12 domain was also partially functional, confirming that receptor dimerization is sufficient to confer proliferative signaling. These results provide the first known example of synthetic ligand-dependent cell proliferation. This approach may have applications for the expansion of genetically modified cells in vitro or in vivo.

This abstract is silent regarding hematopoietic stem cells. What Blau adds to Capon, then, is the ligand-induced proliferation of a murine cell line with a FKBP/EpoR construct induced by FK1201. The instant claims do not relate to the murine cell line, FKBP, EpoR, nor FK1012 of the Blau abstract. Rather, the instant claims recite a chimeric protein derived from thrombopoietin receptor and FKBP_{F36V}, which is dimerized by a defined synthetic divalent homodimer.

Hence, even if Blau supplements Capon by referring to particular constructs, these are *not* the constructs according to the method of the present claims. In other words, the combination of Capon and Blau does not provide a reasonable expectation of success regarding the *particular form* included in the claimed invention: at least one signaling domain derived from a thrombopoietin receptor and at least one drug-binding domain derived from F36V, in the context of rendering a subpopulation of mammalian primary hematopoietic stem cells susceptible to divalent ligand-induced growth, proliferation or differentiation by transducing primary mammalian hematopoietic stem cells with at least one retroviral vector comprising a recombinant

DNA encoding a signaling domain derived from a thrombopoietin receptor fused to an F36V, then exposing the transduced cells to the particular class of claimed divalent ligand effective to induce association of two or more of the encoded fusion proteins such that growth, proliferation or differentiation of said cells is induced, wherein the transduction is carried out *in vivo* or *ex vivo*, and wherein said transduced cells are suitable for introduction into a mammal.

Regarding Ramsjell, the Examiner states on pages 10-11 of the Action, that “Ramsjell et al. further supplements Capon et al and Blau et al. by teaching that TPOR is normally expressed in primitive hematopoietic stem cells and signaling through the TPOR induces the proliferation of hematopoietic stem cells both *in vivo* and *in vitro*.”

The teachings of Ramsjell are rather more complex than the Examiner suggests, and thus only vaguely relate to the instant claims. Indeed, Ramsjell states quite clearly that Tpo **does not**, by itself, induces the proliferation of murine progenitor cells:

To study the ability of Tpo to directly modulate the growth of primitive progenitors, Lin-Sca-1⁺ cells were plated at a concentration of 1 cell/well in complete IMDM supplemented with Tpo (2,000 U/mL), alone or in combination with other cytokines (Table I). *Alone, Tpo induced little or no clonal growth of Lin-Sca-1⁺ cells.* However, Tpo synergized with FL ... (page 4483, col. 1) (emphasis added).

Ramsjell states that “we show that Tpo, unlike Epo, directly and potentially can enhance the growth of a substantial fraction of [primitive] progenitor cells **in combination with other early acting cytokines.**” Page 4481, col. 2-page 4492, col. 1 (emphasis added). In a separate experiment, Ramsjell reports that Tpo alone stimulated growth of 12/300 cells - a 4% increase over control and hardly significant. Page 4485, Figure 4 and accompanying text. What Ramsjell does suggest, on the other hand, is that murine progenitor cells can be expanded *in vitro* with a cocktail of cytokines in which Tpo may act synergistically.

With particular bearing on claim 64, Ramsjell suggests that the Epo receptor, rather than Tpo receptor, would be useful in generating erythrocytes from progenitors:

“despite the corresponding roles of Tpo and Epo as critical and selective regulators of megakaryocytopoiesis and erythropoiesis, respectively, Tpo, but not Epo, appears to affect the growth of primitive multipotent progenitor cells. Therefore, *it appears likely* that, whereas c-mpl is expressed on primitive multipotent progenitors, expression of the *Epo receptor is restricted to progenitors committed to the erythroid* and megakaryocytic lineages.” Page 4490, col. 1 (emphasis added).

Ramsjell thus **teaches away** from the claimed invention in which the mpl receptor expands the population of erythroid cells. Additionally, the data of Ramsjell's Table 3 shows the addition of Tpo did not generate significant erythroid cells:

Table 3. The Effect of SCF and Tpo on Multilineage Colony Formation From Lin⁻ Sca¹⁺ Progenitor Cells in Methylcellulose Cultures

Cytokines	Total Colonies	Colony Distribution, % of Total Colonies						
		E	ME	GM	GEM	GEMe	GEMe	blast
Nil	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SCF	33 (7)	0 (0)	0 (0)	49 (19)	15 (15)	27 (10)	8 (8)	0 (0)
SCF + Tpo	88 (7)	0 (0)	0 (0)	14 (4)	46 (14)	7 (3)	33 (7)	0 (0)

Lin⁻ Sca¹⁺ cells were isolated and plated in methylcellulose cultures as described in Materials and Methods at 500 cells/dish supplemented with Epo and designated cytokines. Cultures were scored for growth of different colony types after 13 to 15 days of incubation at 37°C and 5% CO₂ in air. To confirm the colony phenotype, individual colonies were picked, transferred to a glass slide with a jet air stream, dried, fixed, Giemsa stained and examined by microscopy. Results represent means (± SEM) of four separate experiments, each with duplicate dishes.

In contrast, the claimed invention produced a dramatic differentiation and expansion of erythroid cells when human CD34⁺ cord blood cells were transduced with the mpl/F36V chimeric construct and treated with the divalent ligand of the instant claims: Total cell numbers expanded 175-350-fold, and **90%** of the cells were erythroid after 15 days in culture, in the **absence** of added cytokines (Example 10). Clearly, in comparison with Ramsjell and any of the cited art, this constitutes surprising and unexpected results. Indeed, aside from Ramsjell's teaching away from the use of thrombopoietin receptor to generate erythrocytes, the remaining cited art is silent regarding the expansion of erythroid cells.

Moreover, Ramsjell does not discuss recombinant thrombopoietin receptor, nor retroviruses, nor recombinant chimeric fusion proteins, nor transduced cells, nor any of the details of the recited claims. That Tpo, alone, had a minimal effect on cell expansion and had a synergistic effect with other cytokines does not provide a hint at what has been achieved by the present inventors in the claimed invention.

Regarding Holt, the Examiner asserts, on pages 10-11 of the Action, that Holt supports a § 103 rejection because:

"Holt et al. supplements Capon et al. by teaching various synthetic FKBP dimerizing ligands such as AP103 with improved properties compared to FK1012 and that bind to a modified FKBP with as F36V mutation (Holt et al., column 43, 125-126, 152). Specifically, Holt et al. teaches that FKBP domains have been used in fusion proteins comprising a ligand binding domain and an effector molecule where multimerization of the ligand binding domains in these fusion proteins triggers a desired physiological even such as cell death or cell proliferation (Holt et al., column 2, 75). Holt et al. teaches that the commonly used FKBP dimerizer FK1012 is large, complex and inconvenient to produce

compared to smaller synthetic molecules such as AP1903 (Holt et al, column 1). Holt et al. further teaches that AP1903 has high binding affinity for F36V. (Holt et al., column 43). Holt et al. further teaches effective doses of the dimerizing ligands for both *ex vivo* and *in vivo* dimerization of fusion proteins (Holt et al., column 81, and 150-152).

Holt, in cols. 41-58, presents a “Synthetic Multimerizers Table” which lists **25** divalent multimerizers with corresponding activities. According to these data, the FKBP_{F36V} and FKBP_{F36L} affinity, and Fas killing of, for example, AP1966 is within the same Log value as AP1903. Additionally, AP14272 and AP14283 share similar data with AP1903. In contrast, however, AP1966, AP14272 and AP14283 do **not** share the chemical structure of the instant claims, nor AP1903’s chemical structure.

Moreover, Holt teaches that the divalent multimerizer may be heterodimeric. For example, claims 29-46 support numerous combinations of monomers that may be combined to form a divalent multimerizer. Indeed, there are numerous monomers presented in columns 17-36 (Synthetic Monomers) that have **superior** binding to FKBP mutants than does the monomer (AP1687) that forms divalent AP1903. Additionally, the *in vivo* work of Holt, reflected in Example 3, col. 152, tested for apoptosis in animals using three compounds of Formula IV, which include homodimers and heterodimers (see col. 3), scores of which are beyond the scope of the instant claimed divalent dimerizer.

Regarding FKBP mutants, the Holt patent states that:

FKBP proteins [that] contain one or more amino acid residues corresponding to Tyr25, Phe36, Asp37, Arg42, Phe46, Phe48, Glu54, Val55, or Phe99 of human FKBP12 in which one or more of those amino acid residues are replaced by a difference amino acid, the variant being capable of binding to a compound of this invention. Numerous mutant FKBP domains and fusion proteins containing them ... including by way of illustration, FKBP_s in which phenylalanine at position 36 is replaced with an amino acid having a less bulky side chain, e.g. alanin, valine, methionin or serine. Col. 75, lines 34-45.

This recitation provides at least **81** residue positions suggested for mutation, and at least **4** residue substitutions for each position, such that there are at least **324 possible** substitutions taught by the Holt patent. Moreover, the data presented in columns 17-36 indicate that F36L, F36I, F36M, F36A and F36S (when tested), were within the same Log if not **superior** in binding synthetic monomers compared with F36V. In that regard, Holt teaches away from F36V in favor of those mutants that exhibit superior ligand binding.

Moreover, the differences between the binding affinities of two of the numerous FKBP mutants, FKBP_{F36V} and FKBP_{F36L}, to the homodimeric divalent multimerizers were insubstantial.

Regarding the reference to AP1903 in col. 152, this reads:

As an alternative method for monitoring cell death, cells expressing a construct containing p75 followed by 2 mutant FKBP's followed by a Fas effector domain were transfected using an episomal vector, pCEP4 (Invitrogen Corp.) into which we had inserted DNA encoding hGH under control of a CMV promoter. The vector confers resistance to hygromycin. Clones resistant to hygromycin were selected and tested for response to dimerizer and expression of hGH after treatment with a dimerizer, AP1903. Monitoring Alamar Blue or hGH levels led to very similar EC50 curves for cell viability (measured as a % of control value) plotted against dimerizer concentration. In both cases the concentration of dimerizer corresponding to 50% of control was less than 1 nM.

It is not stated in this Holt paragraph which 2 of the possible 324 FKBP mutants were used in the constructs. It is also unclear whether cells were ever removed from antibiotic selection: thus this data suggests that although cell survival (Alamar Blue staining) corresponded with hGH levels, it does not show that hGH was induced independently of selection pressure. Regardless, Holt does not teach transducing the particular cells of the instant claims, primary hematopoietic stem cells, with the particular construct of the instant claims, a retrovirus comprising a chimeric mpl/F36V construct, and treating those cells with the particular divalent ligand of the present invention to expand or differentiate those cells, particularly the expansion of erythroid cells. Hence, at the time the invention was made, Holt provided only the suggestion to try numerous FKBP mutations and scores of monomeric or dimeric multimerizers with no suggestion or teaching of the particular cells, construct, or treatment of instant claims.

Moreover, it was clear to those of ordinary skill in the art at the time the instant application was filed that established cell lines would not always serve as an adequate indicator of *in vivo* activity or the activity of primary hematopoietic stem cells. In particular, experiments related to growth, proliferation and differentiation in immortalized long-term cultured cells might yield results not applicable to primary cells. The recitation of Lewin to which the Examiner refers on page 13 of the Action is not remediated because Ramsfjell refers to the "natural function of TPOR in primary hematopoietic stem cells" because the dimer of the instant claims is far removed from the "natural function," but rather hematopoietic stem cells transduced by a retroviral vector, expressing a recombinant (un-natural) chimeric mpl/F36V fusion protein, treated with an artificial divalent ligand.

Further, the claimed invention provides for cells that are suitable for introduction into a mammal. Clearly in the context of the claims, the cited art provides little guidance regarding primary mammalian hematopoietic stem cells suitable for introduction into a mammal. Blau refers to *in vitro* studies using the murine IL-3-dependant Ba/F3 cell line. As noted in earlier Replies of record, an IL-3 dependant cell line, in the context of the pending claims, would not be suitable for introduction into a mammal, particularly a human mammal.

In combination, the cited references do not provide an expectation of success regarding the primary hematopoietic stem cells as used in the methods of the claimed invention. Rather, these references provide numerous possible choices or present a new technology or general approach that seemed to be a promising field of experimentation, and give only general guidance as to the particular form of the claimed invention or how to achieve it. Such references do not support an obviousness rejection under the standard set forth by the court in *In re O'Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988).

The court has instructed that although the suggestion to combine references may flow from the nature of the problem, defining the problem in terms of its solution reveals improper hindsight in the selection of the prior art relevant to obviousness. *Ecolchem, Inc. v. S. Cal. Edison Co.*, 227 F.3d 1361, 1372 (Fed. Cir. 2000).

In *Princeton Biochemicals, Inc. v. Beckman Coulter, Inc.*, 411 F.3d 1332, 1337 (Fed. Cir. 2005), the court asked if “an artisan of ordinary skill in the art at the time of the invention, confronted by the same problems as the inventor and with no knowledge of the claimed invention ... have selected the various elements from the prior art and combined them in the manner claimed”? Clearly, based on the cited art, the answer no.

The vast and various teachings of Capon, presented above, combined with Blau's FKBP/mpl construct applied to IL-3-dependent mouse cell line, with Ramsjfell's report on the addition of Tpo and other cytokines to murine cells, and with Holt's multitude of FKBP mutations and dozens of ligands, do not lead one of skill in the art to the particular form of the claimed invention, nor its application to hemopoietic disease or pathological condition in a mammal as recited in the pending claims.

Indeed, the combination fails to provide a “combinati[on of] previously known elements.” *KSR Int'l Co v. Teleflex Inc.*, No. 04-1350 (April 30, 2007) at 14, 15. In summary, comparing the cited references in combination to the claimed invention, it is clear that claimed invention reflects

an advancement and “real innovation.” *KSR Int'l*, at 15. Applicants respectfully request that the § 103 rejection be withdrawn.

CONCLUSION

Applicants respectfully request reconsideration of this application and allowance of the pending claims in view of the above remarks.

Except for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 19-2380. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. §1.136(a)(3).

Respectfully submitted,

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